# Synthesis Based on Affinity Separation (SAS): Separation of Products Having Barbituric Acid Tag from Untagged Compounds by Using Hydrogen Bond Interaction

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**Abstract:** A new method is described for affinity purification of synthetic compounds based on molecular recognition between bis(2,6-diaminopyridine)amide of isophthalic acid and a barbituric acid derivative. The desired compounds possessing the barbituric acid derivative as a tag were readily isolated from the reaction mixture by the following procedure. After each reaction cycle, the reaction mixture was applied to the polystyrene column possessing bis(2,6-diaminopyridine)amide of isophthalic acid as an artificial receptor. The compound possessing the barbituric acid tag was selectively adsorbed on the column, whereas other impurities without the tag such as excess reagents and byproducts were washed off. Subsequent desorption with $CH_2Cl_2$-MeOH (1:1) afforded the desired compound with high purity. This new strategy was applied to the synthesis of a heterocycle, peptides, and oligosaccharides.

**Key words:** affinity purification, molecular recognition, molecular tag, oligosaccharide synthesis, high throughput synthesis

Polymer supported synthesis has greatly simplified work-up and purification procedure of organic reactions to establish combinatorial and parallel synthesis.[1] In solid-phase synthesis, the synthetic product at each step on solid support is immediately separated from a reaction mixture by filtration. Although utility of solid-phase synthesis has been confirmed, there still exist several disadvantages: i) some of conventional reaction conditions in solution are not compatible with reactions on solid-supports, ii) the reaction rates on solid-supports are generally lower than those of the corresponding reactions in solutions. In liquid-phase synthesis using soluble polymer support, the reactivity of compounds on the support is similar to that in solution but additional step, i.e., precipitation of the polymer-supported products, is required for their isolation.[1b] Polymer-supported reagents and scavenger resins have made solution synthesis efficient but this strategy is not always effective for multistep synthesis because of the limited availability of the reagents.[2]

Recently, "tag" methodology has been developed as a hybrid method of solid-phase and solution-phase synthesis.[3] In this methodology, a compound having a tag group is easily separated from untagged molecules by using the affinity of the tag with a respective specific functionality. Several tags such as fluorous affinity tags,[3a-c] hydrophobic tags,[3d-f] a 2-pyridylsilyl tag for acidic extraction,[3g] and an anthracene tag for Diels-Alder resin capture/release[3h] have been reported. The "tag" methodology has advantages over polymer-supported synthesis in the following points. Namely, monitoring of reaction is easy by simple and routine methods such as TLC and HPLC. Additional purification of tagged intermediates is possible by other methods after any reaction steps.

We previously reported a new "tag" strategy, termed "synthesis based on affinity separation (SAS)", in which the desired tagged compound was separated from the reaction mixture by solid-phase extraction using specific molecular recognition (Figure 1).[4] In that work, we employed the interaction between a crown ether (32-crown-10) and ammonium ion for SAS.

In the present study, we investigated a new SAS method by using interaction via multiple hydrogen bonds between a guest residue and its artificial receptor. From many artificial receptors reported, we employed Hamilton's receptor, i.e., bis(2,6-diaminopyridine)amide of isophthalic acid, which forms tight complex with barbituric acid via

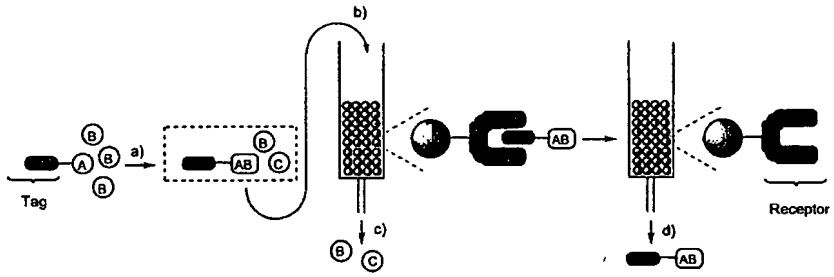



**Figure 1** Synthesis based on affinity separation. A: substrate, B: reagent, AB: product, C: byproduct. a) reaction in solution, b) adsorption of tagged product, c) elution of excess reagent and untagged byproduct, d) desorption of tagged product.

six hydrogen bonds.[5] A barbituric acid moiety was used as a tag and the receptor moiety was bound to polystyrene support (Figure 2).

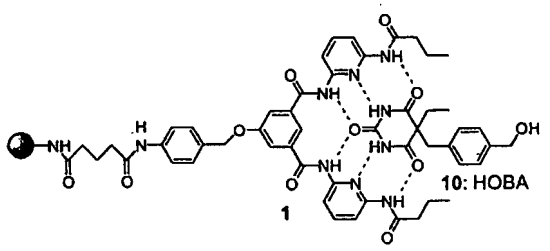
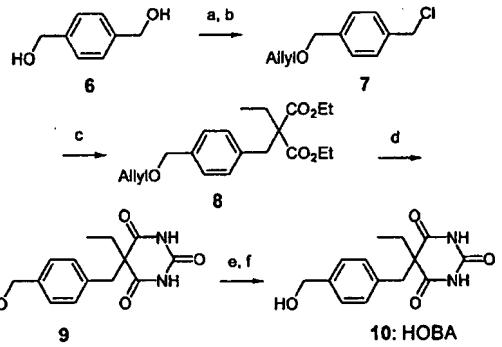



**Figure 2** Host-guest interaction of a polymer-supported receptor with the barbituric acid tag.

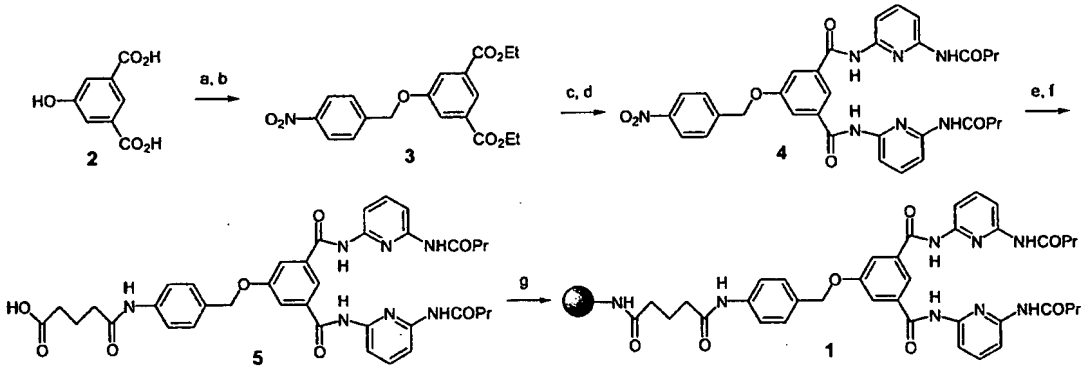
Polymer-supported artificial receptor (**1**) was synthesized from 5-hydroxyisophthalic acid (**2**) as illustrated in Scheme 1.[6] A highly cross-linked macroporous polystyrene resin ArgoPore™–$NH_2$ (1.16 mmol/g) was used as a support since it gave better results than the usual 1% cross-linked gel-type polystyrene resin in the previous study. The barbituric acid tag **10** (HOBA) was synthesized as shown in Scheme 2.[7]

The tagged compounds were effectively retained in the resin column **1** by using nonpolar eluents such as $CH_2Cl_2$, $CHCl_3$, and toluene, which favors the formation of a complex through hydrogen bonds. Since polar solvents disfavor the complex formation, the tagged compounds were readily desorbed from the column by using $CH_2Cl_2$–methanol as an eluent. The separation scale was 0.1 mmol~0.2 mmol for peptides and heterocycle and 0.05 mmol~0.11 mmol for oligosaccharides with 7 g of the resin **1**.

This methodology was first applied to the synthesis of small peptides (Scheme 3).[8] The tag moiety **10** was coupled to the starting Fmoc amino acid **11** by using diisopropylcarbodiimide (DIC) and a catalytic amount of 4-(dimethylamino)pyridine (DMAP) in $CH_2Cl_2$. The reaction mixture was directly applied to the resin column **1**. The tagged **12** was retained in the column, whereas all untagged compounds such as excess **11**, DIC, DMAP, and *N,N'*-diisopropylurea were washed out from the column simply by elution with $CH_2Cl_2$. Elution of **12** with $CH_2Cl_2$-methanol (1:1) and concentration gave purified **12**. Fmoc group in **12** was then removed with 5% piperidine in DMF. Peptide condensation was carried out by the DIC method in $CH_2Cl_2$ to give dipeptide **13**, which was purified by the same affinity separation. After tripeptide **14** was formed in a similar manner, the removal of the Fmoc group, *N*-acetylation, and purification by the affinity separation afforded **15**. The tag moiety of **15** was then removed by transesterification to give **16**, which was separated from the barbituric acid tag **10** by the affinity separation: the desired compound **16** passed through the

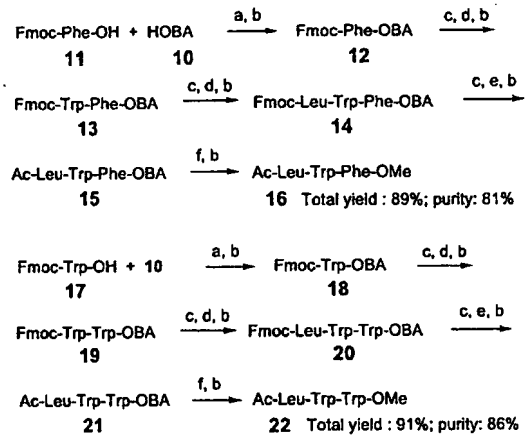



**Scheme 2** Reagents and conditions: a) xylylene glycol (2.0 equiv.), NaH (2.2 equiv.), allylBr (1.0 equiv.), THF, 60°C, 7 h, 63%; b) $[Me_2NCl]^+Cl^-$ (1.3 equiv), MeCN, reflux, 30 min, 99%; c) diethyl ethylmalonate (1.06 equiv.), $KOBu^t$ (1.06 equiv.), toluene, reflux, 6.5h, 98%; d) urea (10 equiv.), NaH (4 equiv.), DMSO, rt, 14 h, 81%; e) $[Ir(COD)(PMePh_2)]^+PF_6^-$ (0.045 equiv.), $H_2$, THF, rt, 100 min, f) $I_2/H_2O$, rt, 30 min, 87%.



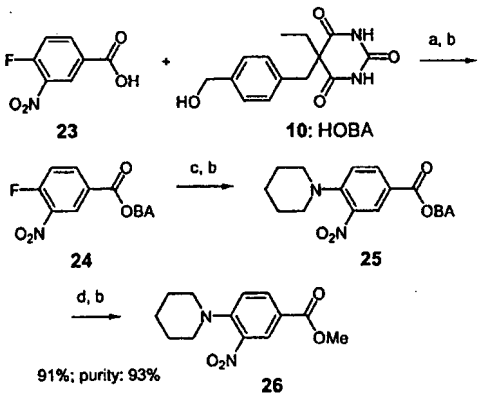

**Scheme 1** Reagents and conditions: a) AcCl, EtOH, reflux, 4 h, 98%; b) *p*-nitrobenzyl bromide (1.12 equiv.), $K_2CO_3$, acetone, reflux, 2 h, 97%; c) n-BuLi (1.95 equiv.), 2,6-diaminopyridine (2.0 equiv.), THF, -78 °C to -60 °C, 8 h, 91%; d) PrCOCl (1.4 equiv.), DIEA, THF, rt, 4 h, 92%; e) $NiCl_2{\cdot}6H_2O$, $NaBH_4$, MeOH, THF, rt, 1.5 h; f) glutaric anhydride (2.0 equiv.), THF, rt, 5 h, 60% over two steps; g) ArgoPore™-$NH_2$ (1.16 mmol/g), DIC, DMAP, DMF, rt, 3 d.

column whereas **10** was adsorbed to the column. The overall yield of the tripeptide **16** was 89% (from HOBA (**10**)) with 81% purity (judged by HPLC analysis at 220 nm). Tripeptide Ac-Leu-Trp-Trp-OMe (**22**) (91% yield with 86% purity) was also synthesized by the same SAS strategy.




**Scheme 3** Reagents and conditions: a) Fmoc-amino acids (1.25 equiv.), DIC (2 equiv.), DMAP, $CH_2Cl_2$; b) affinity separation; c) 5% piperidine in DMF, rt, 8 min; d) Fmoc-amino acids (1.25 equiv.), DIC (2 equiv.), $CH_2Cl_2$; e) $Ac_2O$, TEA, $CH_2Cl_2$; f) 0.2 M NaOMe in MeOH, rt, 10 min.

This new synthetic protocol was then applied to synthesis of *N*-arylpiperidine (Scheme 4). Since 1-methyl-2-pyrrolidone (NMP), which reduces the interaction of the artificial receptor with the tag, was used as a solvent for the aromatic nucleophilic substitution of **24** with piperidine,[9] the reaction mixture was first diluted with $CH_2Cl_2$ and then subjected to the affinity separation. Purification of

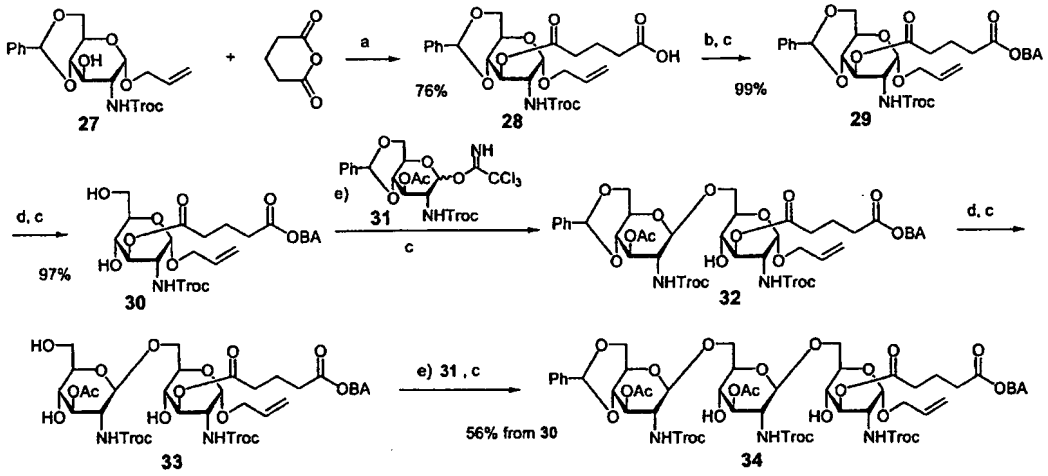



**Scheme 4** Reagents and conditions: a) DIC, DMAP, $CH_2Cl_2$; b) affinity separation; c) piperidine (1.5 equiv.), NMP, rt, 2h; d) 0.2 M MeONa in MeOH, rt, 10 min.

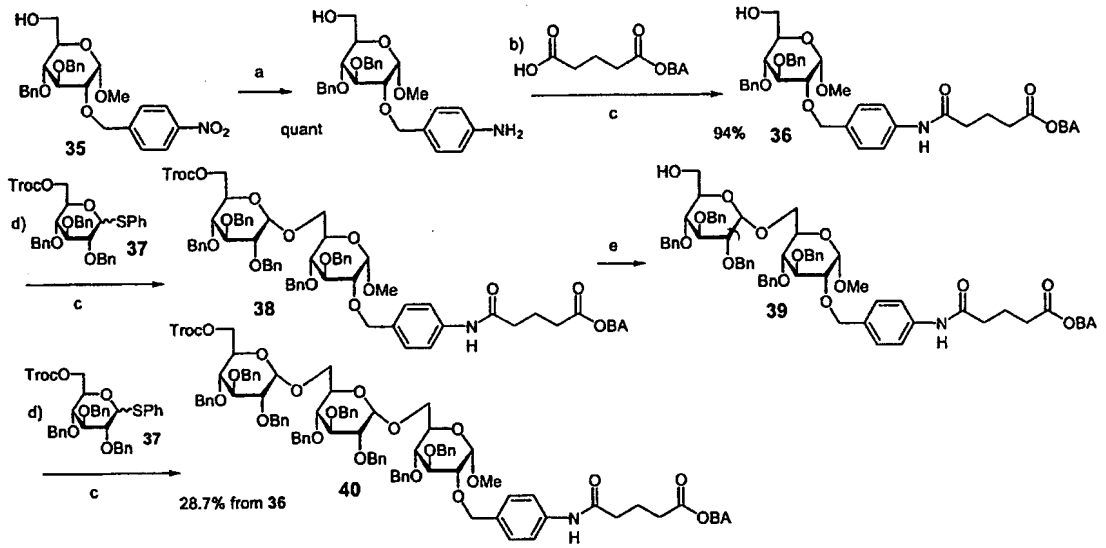
arylpiperidine **25** was thus effected without additional steps for the removal of NMP. Transesterification and isolation by affinity separation gave **26** in overall 91% yield.[10]

We then investigated oligosaccharide synthesis by the present SAS strategy (Scheme 5 and 6). The keys for the success were the following two points: i) the tag moiety was stable under glycosylation conditions and ii) oligosaccharides possessing many protective groups were effectively adsorbed on the resin.

The first example is synthesis of β-(1-6)oligoglucosamine (Scheme 5). The tag **10** was attached to the starting *N*-trichloroethoxycarbonyl (Troc) glucosamine **27** through a glutaric acid spacer. Removal of the benzylidene group of **29** gave a glycosyl acceptor **30**. β-Selective glycosylation of **30** with glycosyl trichloroacetimidate **31** (1.5 equiv. to **30**) was effected by using TMSOTf as a catalyst by virtue




**Scheme 5** Reagents and conditions: a) pyridine, DMAP, $CH_2Cl_2$, 3.5 h; b) HOBA (**10**), DIC, DMAP, $CH_2Cl_2$, 1.5 h; c) affinity separation; d) 5% TFA, 1% $H_2O$ in $CH_2Cl_2$, 0 °C; e) TMSOTf (0.1 equiv.), MS4A, $CH_2Cl_2$, -20 °C, 10 min.



**Scheme 6** Reagents and conditions. a) $NiCl_2 \cdot 6H_2O$, $NaBH_4$, MeOH; b) DIC, $CH_2Cl_2$; c) affinity separation; d) PhIO, TMSOTf, $Et_2O$/dioxane; e) Zn-Cu couple, AcOH.

of the neighboring participation of the *N*-Troc group to afford the disaccharide **32**.[11] Removal of the benzylidene from **32** followed by glycosylation with **31** (5 equiv.) gave trisaccharide **34**. After each reaction step, the product was rapidly separated from the reaction mixture by the affinity separation in this synthesis, too. Since there are two and three hydroxy groups in acceptors **30** and **33**, respectively, some undesired byproducts were formed by their glycosylation at more hindered 4-positions. These byproducts were not separated by affinity separation like solid-phase synthesis, since they had the tag moiety. The desired trisaccharide **34** was, however, readily purified by additional simple silica-gel chromatography. The purified **34** was thus obtained in 56% yield from **30** after silica-gel column chromatography.[12] In the case of solid-phase synthesis, purification is only possible after products are cleaved from solid-supports. By using the present SAS method, products can be purified by affinity separation as well as other purification methods. Purified synthetic intermediates can be subjected to next reaction. This is important feature of the present methodology.

As shown in Scheme 6, oligosaccharide synthesis was carried out under the conditions for α-selective glycosylation by virtue of the solvent effect of ether using 2-*O*-benzylated thioglycosyl donors. Since the solubilities of glycosyl acceptors having the barbituric acid tag in ether were low, a mixture of ether-dioxane was used as the reaction solvent.[13] We previously found combinations of iodosobenzene (PhIO) and various acids effectively promote glycosylation using thioglycosyl donors.[14] Among those found to be satisfactory, the combination of PhIO and TMSOTf was employed in the present study.[15]

The tagged acceptor **36** was prepared from 4-nitrobenzylglucoside **35** via reduction of the nitro group followed by coupling with the tag moiety (Scheme 6).[16] Glycosylation of the tagged acceptor **36** with excess phenyl 6-*O*-Troc-thioglycoside **37** (1.5 equiv.) using PhIO and TMSOTf gave disaccharide **38** (α:β = 10:1).[17] The 6-*O*-Troc group of **38** was removed and the resulting 6-*O*-free disaccharide **39** was then glycosylated with **37** to give the trisaccharide **40** (28.7% from **36**).[18] After each reaction step, the product was rapidly separated from the reaction mixture by the affinity separation. Since the acylaminobenzyl linker was partly cleaved at the glycosylation step, the final product **40** was purified by silica-gel column chromatography after the affinity separation.

In summary, we have developed a new synthetic strategy based on affinity separation. The present method can be applied to the synthesis of relatively large molecules such as protected oligosaccharides owing to the strong affinity of the barbituric acid tag with its artificial receptor, whereas previous tag strategies have been applied to synthesis of small molecules.[19] The strategy greatly facilitates the purification procedures in solution-phase synthesis and is expected to be particularly useful for multiple parallel synthesis and combinatorial library preparation where application of solid-phase synthesis is difficult.

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(6) The experimental procedure for the preparation of the polymer supported receptor **1**.
Diethyl 5-(4-Nitrophenylmethyloxy)isophthalate (**3**). The mixture of diethyl 5-hydroxyisophthalate (7.68 g, 32.2 mmol), *p*-nitrobenzyl bromide (7.80 g, 36.0 mmol), and $K_2CO_3$ (8.0 g, 58 mmol) in acetone (100 ml) was refluxed for 2 h. After the mixture was cooled to room temperature, insoluble materials were removed by filtration and the filtrate was concentrated in vacuo. The crystalline residue was recrystallized from ethyl acetate and hexane to give **3** (11.6 g, 96.5%). ESI-MS (positive) m/z 396.1 [$(M+Na)^+$].
*N,N'*-Bis[2-(6-aminopyridyl)]-5-(4-nitrophenyl-methyloxy)isophthaldiamide. To a solution of 2,6-diaminopyridine (8.00 g, 73.3 mmol) in anhydrous THF (150 ml) was added 25.0 ml of n-butyl lithium in hexane (2.52 mol·l$^{-1}$) at −78 °C under $N_2$. After the mixture was stirred for 20 min, a solution of **3** (6.00 g, 16.1 mmol) in anhydrous THF (55 ml) was added dropwise. The mixture was stirred at −78 °C to −60 °C for 6 h and water (30 ml) was added to quench the reaction. After addition of EtOAc (200 ml), the organic layer was washed with water and brine, dried over $Na_2SO_4$. Parts of the desired product were crystallized from extracts. Filtration afforded 6.40 g of the product. The filtrate was concentrated in vacuo and the residue was purified by silica-gel column chromatography (EtOAc/hexane = 1:1) to give another 0.90 g of the product. Yield: 7.30 g (90.9%). ESI-MS (positive) m/z 500.2 [$(M+H)^+$].
*N,N'*-Bis[2-(6-butyrylaminopyridyl)]-5-(4-nitrophenylmethyloxy)isophthaldiamide (**4**). To a mixture of *N,N'*-bis[2-(6-aminopyridyl)]-5-(4-nitrophenyl-methloxy)-isophthaldiamide (3.32 g, 3.35 mmol) and *N,N*-diisopropylethylamine (2.5 ml, 14.4 mmol) in THF (80 ml) was added butanoyl chloride (2.0 ml, 19.3 mmol). The mixture was stirred at room temperature for 4 h under $N_2$. After addition of water (50 ml) and EtOAc (200 ml), the organic layer was washed with 0.5 M NaOH (50 ml × 1) and brine (100 ml × 2), dried over $Na_2SO_4$, and concentrated. The residue was purified by silica-gel column chromatography ($CHCl_3$/acetone = 10:1) to give **4** (3.8 g, 91.8%). ESI-MS (positive) m/z 662.3 [$(M+Na)]^+$. $^1$H NMR (600 MHz, DMSO), δ = 10.50 (2H, s, 2 × CONH), 10.09 (2H, s, 2 × CONH), 8.30 (2H, d, *J* = 8.5 Hz, $PhNO_2$), 8.16 (1H, s, Ph), 7.83~7.75 (10H, m, Ar-H), 5.47 (2H, s,-$OCH_2$-), 2.37 (4H, t, *J* = 7.3 Hz, 2 × -$COCH_2$-), 1.59 (4H, m, 2 × $CH_2$), 0.90 (6H, t, *J* = 7.3 Hz, 2 × $CH_3$).
*N,N'*-Bis[2-(6-butyrylaminopyridyl)]-5-[4-(4-carboxyl-butyryl)phenylmethyloxy]isophthaldiamide (**5**). To a solution of $NiCl_2$·$6H_2O$ (0.3 g, 1.26 mmol) in MeOH (80 ml) was added of $NaBH_4$ (0.1 g, 2.64 mmol) (the color of solution changed from green to black) and a solution of **4** (2.0 g, 3.13 mmol) in THF (40 ml) was added. $NaBH_4$ (4.0 g, 106 mmol) was then added to the mixture by portions for 1.5 h. After that 1M NaOH (20 ml) and EtOAc (80 ml) were added, the organic layer was washed with brine (60 ml × 3), dried over $Na_2SO_4$, and concentrated in vacuo. To the solution of the residue in THF (30 ml) was added glutaric anhydride (1.0 g, 8.77 mmol) and the mixture was stirred at room temperature for 5 h. The desired **5** precipitated was collected by filtration and washed with THF to give 1.10 g of **5**. The filtrate was concentrated and the residue was purified by silica-gel column chromatography ($CHCl_3$/MeOH = 20:1) to give another 0.25 g of the product. Yield 1.35 g (59.7%). ESI-MS m/z 722.3 (negative) [$(M-H)^-$]. $^1$H NMR (600 MHz, DMSO), δ = 10.49 (2H, s, 2 × CONH), 10.11 (2H, s, 2 × CONH), 10.00 (1H, s, CONH), 8.13(1H, s, Ph), 7.86-7.72 (5H, Ar-H),7.62 (2H, d, *J* = 7.7 Hz, *Ph*NHCO), 7.42 (2H, d, *J* = 7.7 Hz, *Ph*NHCO), 5.21(2H, s, -$OCH_2$-), 2.37 (6H, m, 3 x $CH_2CO$), 2.26 (2H, t, $CH_2CO$), 1.75 (4H, m, 2 × $CH_2$), 1.61 (4H, m, 2 × $CH_2$), 0.90 (6H, t, *J* = 6.9 Hz, 2 × $CH_3$).
Preparation of the polymer supported receptor **1**. To a suspension ArgoPore-$NH_2$-™ (1.16 mmol/g) (1.00 g, 1.16 mmol of $NH_2$ group), **5** (1.25 g, 1.73 mmol), and DMAP (5.0 mg, 0.04 mmol) in DMF (10 ml) was added DIC (545 µl, 3.48 mmol) at room temperature. After 3 d of agitation, the resin was washed with DMF (20 ml × 3), $CH_2Cl_2$-MeOH 1:1 (20 ml × 3), and $CH_2Cl_2$ (20 ml × 3) and dried under reduced pressure to give the receptor-bound resin (1.42 g, 50.2%).

(7) The experimental procedure for the preparation of the tag **10**.
4-Allyloxymethylbenzyl Alcohol. To a solution of *p*-xylylene glycol (**6**) (11.1 g, 80.3 mmol) in anhydrous THF (200 ml) was added NaH (60% oil suspension, 3.52 g, 88.0 mmol). The mixture was stirred at room temperature for 1 h. Allyl bromide (3.46 ml, 40.0 mmol) was added to the solution and the mixture was stirred at 60 °C for 7 h. After addition of EtOAc and aqueous saturated citric acid solution, the organic layer was washed with water and brine, dried over $Na_2SO_4$, and concentrated in vacuo. The residue was purified by silica-gel column chromatography ($CHCl_3$/acetone = 1:0 to 9:1) to give 5.64 g (63.4%) of the desired product. ESI-MS (positive) m/z 178.44 ($M^+$); 196.44 [$(M+H_2O)^+$].
4-Allyloxymethylbenzyl Chloride (**7**). To a solution of chloromethylenedimethylammonium chloride (1.30 g, 10.2 mmol) in anhydrous $CH_3CN$ (10 ml) was added dropwise a solution of 4-allyloxyphenylmethanol (1.30 g, 7.29 mmol) in anhydrous $CH_3CN$ (10 ml) under $N_2$ and the mixture was refluxed for 40 min. After the mixture was cooled to room temperature, ice-cold water (10 ml) was added. The mixture was extracted with EtOAc and the organic layer was washed with water, dried over $K_2CO_3$, and concentrated in vacuo to give **7** (1.14 g, 98.6%).
Diethyl 2-(4-Allyloxymethylbenzyl)-2-ethylmalonate (**8**). To a solution of diethyl ethylmalonate (2.30 g, 12.2 mmol) in anhydrous toluene (40 ml) was added potassium t-butoxide (1.41 g, 12.6 mmol) under $N_2$. After the mixture was refluxed for 30 min, the solution of **7** (1.40 g, 7.12 mmol) in toluene (20 ml) was added. The mixture was refluxed for 6.5 h and then cooled to room temperature. After addition of water and EtOAc, the organic layer was washed with 0.5 M HCl, saturated $NaHCO_3$ solution, and brine, dried over $Na_2SO_4$, and

concentrated in vacuo. The residue was purified by silica-gel column chromatography (hexane/EtOAc = 20:1 to 10:1) to afford **8** (3.90 g, 97.7%). ESI-MS (positive) m/z 349.35 [$(M+H)^+$, 18%]; 366.35 [$(M+H_2O)^+$, 40%]; 371.30 [$(M+Na)^+$, 100%). $^1$H NMR (500 MHz, $CDCl_3$), δ = 7.22 (2H, d, *J* = 7.7 Hz, Ar-H), 7.07 (2H, d, *J* = 8.0 Hz, Ar-H), 5.94 (1H, m, $CH_2$=C*H*-$CH_2$-), 5.29 (1H, m, C$H_2$=CH-$CH_2$-), 5.19 (1H, m, C$H_2$=CH-$CH_2$-), 4.47 (2H, s, -OC$H_2$PhC$H_2$-), 4.18 (4H, m, 2 × -OC$H_2$$CH_3$), 4.01 (2H, m, $CH_2$=CH-C$H_2$-), 3.23 (2H, s, -$OCH_2$PhC$H_2$-), 1.82 (2H, q, *J* = 7.6 Hz, -C$H_2$$CH_3$), 1.24 (6H, t, *J* = 7.1 Hz, 2 × -$OCH_2$C$H_3$), 0.92 (3H, t, *J* = 7.6 Hz, -$CH_2$C$H_3$).

5-Ethyl-5-(4-allyloxymethylbenzyl)barbituric Acid (**9**). To a solution of urea (7.00 g, 117 mmol) in DMSO (25 ml) was added NaH (60% oil suspension, 2.0 g, 50 mmol) under $N_2$. After the mixture was stirred at room temperature for 30 min, **8** (4.10 g, 11.8 mmol) was added. The mixture was stirred at room temperature for 14 h and then poured into 20 g of ice. The mixture was acidified with 2M HCl and then extracted with EtOAc. The organic layer was washed with water, dried over $Na_2SO_4$, and concentrated in vacuo. The residue was purified by silica-gel column chromatography (hexane/EtOAc = 3:1 to 2:1) to give **9** (3.00 g, 80.6%). ESI-MS (positive) m/z 339.15 [$(M+Na)^+$]. $^1$H NMR (600 MHz, $CDCl_3$), δ = 7.86 (2H, s, NH), 7.22 (2H, d, *J* = 7.7 Hz, Ar-H), 7.08 (2H, d, *J* = 8 Hz, Ar-H), 5.93 (1H, m, $CH_2$=C*H*-$CH_2$-), 5.29 (1H, m, C$H_2$=CH-$CH_2$-), 5.20 (1H, m, C$H_2$=CH-$CH_2$-), 4.46 (2H, s, -OC$H_2$PhC$H_2$-), 4.00 (2H, m, $CH_2$=CH-C$H_2$-), 3.27(2H, s, -$OCH_2$PhC$H_2$-), 2.20 (2H, q, *J* = 7.6 Hz, -C$H_2$$CH_3$), 0.92 (3H, t, *J* = 7.6 Hz, -$CH_2$C$H_3$).

5-Ethyl-5-(4-hydroxymethylbenzyl)barbituric Acid (**HOBA**) (**10**). To a degassed solution of **9** (2. 89 g, 9,13 mmol) in anhydrous THF (100 ml) was added $[Ir(COD)(PMePPh_2)]^+PF_6^-$ (350 mg, 0.045 equiv) and the solution was stirred under $H_2$ at room temperature for 5 min (the color of the solution changed from red to yellow) and then for 100 min under $N_2$. Water (45 ml) and iodine (4.4 g) was added to the mixture successively. After the mixture was stirred for 30 min, aqueous 10% $Na_2S_2O_3$ solution (10 ml) was added to quench the reaction. The mixture was extracted with EtOAc and the organic layer was washed with brine, dried over $Na_2SO_4$, and concentrated in vacuo. The residue was purified by silica-gel column chromatography ($CHCl_3$/MeOH = 20:1) to give **10** (2.20 g, 87.3%). ESI-MS (positive) m/z 575.3 [$(2M+Na)^+$]; 315.1 [$(M+K)^+$]; 299.2 [$(M+Na)^+$]. $^1$H NMR (600MHz, DMSO), δ = 7.18 (2H, d, *J* = 8 Hz, Ar-H), 6.94 (2H, d, *J* = 8 Hz, Ar-H), 5.15 (t, 1H, OH), 4.24 (2H, m, -OC$H_2$PhC$H_2$-), 3.07 (2H, s, -$OCH_2$PhC$H_2$-), 1.97 (2H, q, *J* = 7.6 Hz, -C$H_2$$CH_3$), 0.76 (3H, t, *J* = 7.6 Hz, -$CH_2$C$H_3$).

(8) General procedure for affinity separation. After completion of the reaction, the reaction mixture was directly applied to the resin **1** column (7.0 g: 1.5 cm × 7 cm, $CH_2Cl_2$) unless otherwise noted. After untagged compounds were washed off with $CH_2Cl_2$ (70 ml), the tagged compound was eluted with MeOH-$CH_2Cl_2$ (1:1) (30 ml). Evaporation of the solvents afforded the desired product having the tag.

(9) Dankwardt, S. M.; Newman, S. R.; Krstenansky, J. L. *Tetrahedron Lett.* **1995**, *36*, 4923.

(10) The experimental procedure for the preparation of methyl 4-piperidino-3-nitrobenzoate (**26**). To a solution of HOBA (**10**) (27.6 mg, 0.100 mmol), 4-fluruo-3-nitrobenzoic acid (22.0 mg, 0.119 mmol), and DMAP (1mg, 8 μmol) in anhydrous $CH_2Cl_2$ (3 ml) was added DIC (30 μl, 0.19 mmol). The mixture was stirred at room temperature for 2 h and then applied to the affinity separation to give 5-ethyl-5-[4-(4-fluruo-3-nitrobenzoyl)oxymethylbenzyl]barbituric acid (**24**). To a solution of **24** in *N*-methylpyrrolidone (NMP) (0.8 ml) was added piperidine (20 μl, 0.20 mmol). The mixture was stirred at room temperature for 2 h, diluted with $CH_2Cl_2$ (8 ml), and then applied to the affinity separation. The desired 5-ethyl-5-[4-(4-piperidino-3-nitrobenzoyl)oxymethylbenzyl]barbituric acid (**25**) was eluted at both $CH_2Cl_2$ and $CH_2Cl_2$-MeOH (1:1) fractions. The $CH_2Cl_2$ fraction containing **25** was concentrated and then applied to the affinity separation again. The $CH_2Cl_2$-MeOH (1:1) fractions were combined and concentrated. Compound **25** thus obtained was dissolved in 0.2 M MeONa in MeOH (2.0 ml) and the mixture was stirred at room temperature for 30 min. After addition of AcOH (3 drops), the mixture was concentrated in vacuo. The residue was dissolved in $CHCl_3$ and then applied to the affinity separation. The desired **26** was eluted with $CHCl_3$, whereas HOBA (**10**) was eluted with $CH_2Cl_2$/MeOH (1:1). Evaporation of the solvent afforded **26** (24.0 mg, 90.9%). ESI-MS (positive) m/z 265.2 [$(M+H)^+$]. $^1$H NMR (600 MHz, $CDCl_3$), δ = 8.42 (1H, s, Ar-H), 8.02 (1H, d, *J* = 8.8 Hz, Ar-H), 7.05 (1H, d, *J* = 8.8 Hz, Ar-H), 3.90 (3H, s, $CH_3$), 3.15 (4H, t, $NCH_2$), 1.72 (4H, m, $CH_2$), 1.51 (2H, m, $CH_2$).

(11) Liu, W.-C.; Oikawa, M.; Fukase, K.; Suda, Y.; Kusumoto, S. *Bull. Chem. Soc., Jpn.* **1999**, *72*, 1377.

(12) The experimental procedure for the preparation of trisaccharide **34**. The mixture of the glycosyl acceptor **30** (69.2 mg, 0.09 mmol), the glycosyl donor **31** (85 mg, 0.135 mmol), and MS 4A in $CH_2Cl_2$ (4 ml) was stirred at room temperature for 1 h under $N_2$ atmosphere and then cooled to −20 °C. TMSOTf (4.0 μl, 22 μmol) was added and the mixture was stirred at the same temperature for 10 min. The mixture was directly applied to the affinity separation to give disaccharide **32**. Disaccharide **32** was dissolved in $CH_2Cl_2$ (4 ml) containing 5% TFA and 1% water. The mixture was stirred at 0 °C for 30 min, diluted with EtOAc (15 ml), washed with saturated $NaHCO_3$ solution and brine, dried over $Na_2SO_4$ and concentrated. The residue was applied to the affinity separation to afford disaccharide acceptor **33**. The mixture of **33** (59.2 mg, 0.0517 mmol), **31** (161 mg, 0.256 mmol), and MS 4A in $CH_2Cl_2$ (5 ml) was stirred at room temperature for 1 h under $N_2$, and then cooled to −20°C. TMSOTf (4.0 μl, 22 μmol) was added and the mixture was stirred at the same temperature for 10 min. The mixture was directly applied to the affinity separation to give 80.1 mg of the crude product, which was then purified by silica-gel column chromatography to afford the pure trisaccharide **34** (47.0 mg, 56.0%). ESI-MS m/z 1630.0 [$(M+Na)^+$]. $^1$H NMR (500 MHz, $CDCl_3$), δ = 8.45 (2H, NH: barbituric acid), 7.45 (2H, m, Ph-H), 7.34 (3H, m, Ph-H), 7.20 (2H, d, *J* = 7.6 Hz, Ph-H), 7.09 (2H, d, *J* = 8.1 Hz, Ph-H), 6.28 (1H, br s, 2-N*H*: $GlcN_C$), 5.92 (1H, m, $CH_2$=CH-$CH_2$-), 5.51 (1H, s, PhC*H*=), 5.43 (1H, d, *J* = 9.9 Hz, 2-N*H*: $GlcN_A$), 5.37 (1H, br s, 2-NH: $GlcN_B$), 5.33 (1H, m, $CH_2$=CH-$CH_2$-), 5.24 (1H, m, C$H_2$=CH-$CH_2$-), 5.18 (1H, t, *J* = 9.7 Hz, H-3: $GlcN_A$), 5.13 (1H, t, *J* = 9.9 Hz, H-3: $GlcN_C$), 5.05 (1H, H-3: $GlcN_B$), 5.02 (1H, d, *J* = 14 Hz, -OC$H_2$PhC$H_2$-), 4.97 (1H, d, *J* = 14 Hz, -OC$H_2$PhC$H_2$-). 4.90 (d, *J* = 3.7 Hz, 1H, H-1: $GlcN_A$), 4.79-4.65 (6H, $CCl_3CH_2$- × 3), 4.65 (1H, d, *J* = 8.3 Hz, H-1: $GlcN_C$), 4.63 (1H, d, *J* = 8.6 Hz, H-1: $GlcN_B$), 4.37 (1H, dd, *J* = 5.6 Hz, 10.6 Hz, H-6: $GlcN_C$), 4.22 (1H, m, $CH_2$=CH-C$H_2$-), 4.21 (1H, H-6: $GlcN_B$), 4.17 (1H, H-6: $GlcN_A$), 4.02 (1H, m, $CH_2$=CH-C$H_2$-), 3.96 (1H, H-5: $GlcN_A$), 3.95 (1H, H-2: $GlcN_A$), 3.84 (1H, H-6': $GlcN_A$), 3.82 (1H, H-6': $GlcN_C$), 3.80 (1H, H-2: $GlcN_C$), 3.79 (1H, H-6': $GlcN_B$), 3.71 (1H, H-4: $GlcN_C$), 3.70 (1H, H-4: $GlcN_A$), 3.62 (1H, H-2: $GlcN_B$), 3,53 (1H, H-5: $GlcN_B$), 3.52 (1H, H-5: $GlcN_C$), 3.51 (1H, H-4: $GlcN_B$), 3.26 (2H, m, -$OCH_2$PhC$H_2$-), 2.35-2.22 (4H, m, -COC$H_2$$CH_2$C$H_2$CO-), 2.18 (2H, q, *J* = 6.2 Hz, $CH_3$C$H_2$-), 2.10 (3H, s, C$H_3$CO-), 1.90 (2H, m, -

$COCH_2CH_2CH_2CO$-), (3H, t, $J$ = 6.2 Hz, $CH_3CH_2$-) (the saccharide residues are designated as $GlcN_A$, $GlcN_B$, and $GlcN_C$ (glucosamine residue) from reducing end).

(13) α-Promoting solvent effect of dioxane was reported. a) Demchenko, A. V.; Rousson, E.; Boons, G.-J. *Tetrahedron Lett.* **1999**, *40*, 6523. b) Rademann, J.; Schmidt, R. R. *J. Org. Chem.* **1997**, *62*, 3650.

(14) a) Fukase, K.; Kinoshita, I.; Kanoh, T.; Nakai, Y.; Hasuoka, A.; Kusumoto, S. *Tetrahedron* **1996**, *52*, 3897. b) Fukase, K.; Nakai, Y.; Kanoh, T.; Kusumoto, S. *Synlett* **1998**, 84.

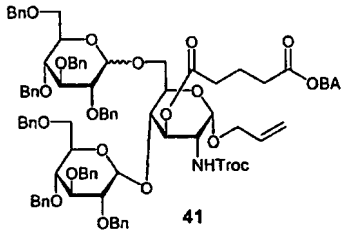
(15) α-Selectivity is expected to be increased by using combinations of PhIO with $SnCl_2$-$AgClO_4$, $SnCl_4$-$AgClO_4$, $BiCl_3$-$AgClO_4$, and $SbCl_3$-$AgClO_4$, though these reagents require careful handling.[14]

(16) An acylaminobenzyl linker, which can be cleaved by DDQ oxidation, was used for solid-phase oligosaccharide synthesis. Fukase, K.; Nakai, Y.; Egusa, K.; Porco Jr., J. A.; Kusumoto S. *Synlett* **1999**, 1074.

(17) We previously reported α-orienting effect of the 6-*O*-Troc group.[14] Fukase, K.; Yoshimura, T.; Kotani, S.; Kusumoto S. *Bull. Chem. Soc. Jpn.* **1994**, *67*, 473.

(18) The experimental procedure for the preparation of trisaccharide **40**. The mixture of the glycosyl acceptor **36** (97.4 mg, 0.114 mmol), phenyl thioglucoside **37** (122.8 mg, 0.171 mmol), PhIO (48 mg, 0.22 mmol), and MS 4A in diethyl ether (5.0 ml) and dioxane (4.0 ml) was stirred for 1 h at r.t. under $N_2$ then cooled to −10 °C. TMSOTf (20 μl, 0.110 mmol) was added and the reaction mixture was stirred for 1 h. The reaction was quenched by addition of aqueous saturated $NaHCO_3$ solution and ethyl acetate. The organic layer was washed with brine, dried over $Na_2SO_4$, and concentrated. The residue was dissolved in $CH_2Cl_2$ and then subjected to the affinity separation. Since the product **38** contained small amount of the starting material **36**, the mixture of **38** and **36** was subjected repeatedly to glycosylation and then affinity separation to give **38** (α:β = 10:1). ESI-MS m/z 1480.4 [$(M+Na)^+$]; $^1$H NMR (600 MHz, $CDCl_3$), α-anomer: δ = 8.07 (2H, NH: barbituric acid), 4.95 (1H, d, $J$ = 3.6 Hz, H-1: $Glc_B$), 4.53 (1H, d, $J$ = 3.6 Hz, H-1: $Glc_A$), 4.32 (2H, d, $J$ = 3.3 Hz, H-6: $Glc_B$), 3.972 (1H, dd, $J$ = 9.3 Hz, 9.3 Hz, H-3: $Glc_A$), 3.966 (1H, dd, $J$ = 9.1 Hz, 9.1 Hz, H-3: $Glc_B$), 3.86 (m, 1H, H-5: $Glc_B$), 3.79 (1H, m, H-6, $Glc_A$), 3.77 (1H, m, H-5: $Glc_A$), 3.71 (1H, H-6': $Glc_A$), 3.64 (1H, dd, $J$ = 9.1 Hz, 9.3 Hz, H-4: $Glc_A$), 3.50 (1H, H-2: $Glc_B$), 3.50 (1H, H-4: $Glc_B$), 3.40 (1H, dd, $J$ = 3.6 Hz, 9.6 Hz, H-2: $Glc_A$), 3.34 (3H, s, $-OCH_3$), 3.257 (2H, s, $-OCH_2PhCH_2$-), 2.48-2.30 (4H, $-COCH_2CH_2CH_2CO$-), 2.19 (2H, $CH_3CH_2$-), 2.0 (2H, m, $-COCH_2CH_2CH_2CO$-), 0.92 (3H, $CH_3CH_2$-). β-anomer: δ = 4.60 (1H, H-1: $Glc_A$), 4.35 (1H, d, $J$ = 8.4 Hz, H-1: $Glc_B$), 3.44 (1H, H-2: $Glc_A$), 3.31 (3H, S, $-OCH_3$). To a solution of **38** in acetic acid (1 ml) was added Zn-Cu (260 mg) and the mixture was stirred at r.t. for 1 h. Ethyl acetate was added and the insoluble materials were removed by filtration. The filtrate was washed with aqueous saturated $NaHCO_3$ solution and brine, dried over $Na_2SO_4$, and concentrated. The residue was then subjected to the affinity separation to give **39**. The mixture of **39**, phenyl thioglucoside **37** (105.4 mg, 0.147 mmol), PhIO (48 mg, 0.22 mmol), and MS 4A in diethyl ether (4.0 ml) and dioxane (2.0 ml) was stirred at r.t. for 1 h under $N_2$ and then cooled to −10 °C. TMSOTf (20 μl, 0.110 mmol) was added and the reaction mixture was stirred for 1 h. The reaction was quenched by addition of aqueous saturated $NaHCO_3$ solution and ethyl acetate. The organic layer was washed with brine, dried over $Na_2SO_4$, and concentrated. The residue was dissolved in $CH_2Cl_2$ and then subjected to the affinity separation to give 119.2 mg of the crude product. The product was purified by silica-gel column chromatography to afford compound **40** (62.0 mg, 28.7%). ESI-MS m/z 1912.6 [$(M+Na)^+$]; $^1$H NMR (600 MHz, $CDCl_3$), α(1-6)α(1-6)α: δ = 8.12 (2H, NH: barbituric acid), 4.98 (1H, d, $J$ = 3.3 Hz, H-1: $Glc_C$), 4.90 (1H, H-1: $Glc_B$), 4.54 (1H, H-1: $Glc_A$), 4.30 (2H, H-6: $Glc_C$), 3.96 (1H, dd, $J$ = 9.3Hz,9.3 Hz, H-3: $Glc_C$), 3.95 (1H, H-3: $Glc_A$), 3.94 (1H, H-3: $Glc_B$), 3.81 (m, 1H, H-5: $Glc_C$), 3.77 (1H, m, H-6, $Glc_A$), 3.76 (1H, m, H-6: $Glc_B$), 3.76 (1H, m, H-5: $Glc_B$), 3.72 (1H, H-5: $Glc_A$), 3.70 (1H, H-4: $Glc_B$), 3.66 (1H, H-4, $Glc_A$), 3.48 (1H, H-2: $Glc_C$), 3.48 (1H, H-4: $Glc_C$), 3.38 (1H, dd, $J$ = 3.0 Hz, 9.3 Hz, H-2: $Glc_A$), 3.37 (1H, dd, $J$ = 3.0 Hz, 9.3 Hz, H-2: $Glc_B$), 3.32 (3H, s, $-OCH_3$), 3.24 (2H, s, $-OCH_2PhCH_2$-), 2.48-2.30 (4H, $-COCH_2CH_2CH_2CO$-), 2.19 (2H, $CH_3CH_2$-), 2.01 (2H, m, $-COCH_2CH_2CH_2CO$-), 0.91 (3H, t, $J$ = 9.1 Hz, $CH_3CH_2$-).

(19) The trisaccharide **41** did not exhibit sufficiently strong affinity to the receptor and leaked from the resin column when $CH_2Cl_2$ was used as an eluent. Large hydrophobic moiety in **41** decreases the relative affinity of the barbituric acid tag with the receptor. When less polar toluene, in which the affinity is expected to increase, was used as an eluent, **41** was effectively retained in the column 1, which was then washed successively with toluene and $CH_2Cl_2$. Compound **41** was not eluted during the washing. Elution with $CH_2Cl_2$-methanol (1:1) afforded **41**.




**41**

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